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A systematic review of *LDLR, PCSK9,* and *APOB* variants in Asia

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HIGHLIGHTS

- This systematic review provides information on *LDLR, APOB, PCSK9* variants in Asia.
- Among 8994 FH families in 48 Asian countries, 629 *LDLR* variants were reported.
- Twenty variants were reported as the common variants in Asia.
- The frequency and distribution of the variants were high in East Asia.

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ABSTRACT

Background and aims: Genetic identification is a public health care concern for management of familial hypercholesterolemia (FH) associated cardiovascular morbidity and mortality. This study presents the spectrum and distribution of *LDLR, APOB, PCSK9* gene mutations in Asia.
Methods: Databases were searched for English papers from 1950 to 2019. The spectrum of the variants was investigated in 8994 FH families in 48 Asian countries. We determined the frequency of variants, zygosity, and clinical features.
Results: Twenty countries have studied *LDLR* variants. A total of 629 mutations were reported and twenty variants were accounted as common variants in different populations. China, Japan, India and Taiwan constituted 68% of published articles. The most frequent mutation was reported in Japan but was not common in other countries. Other missense variants accounted for 50% of the mutations, frameshifts 19%, and nonsense 11%. The pooled frequency of variation was estimated in 1867 individuals. Approximately 67% of Iranian families were homozygous.,The common variant was p.Ser130Ter. p.Arg3527Trp in *APOB* was common among 184 heterozygous patients; the common variant of *PCSK9* was p.Glu32Lys.
Conclusions: This is the first systematic review of *LDLR, APOB, PCSK9* mutations in FH patients in Asia. These findings underscore the need to fill in the gap of studies on different populations in Asia. It also underlies the importance of early detection and management to decrease atherosclerosis and cardiovascular risk in different ethnicities.

1. Introduction

Pathogenic variants of the *LDLR* gene, as the main cause of familial hypercholesterolemia (FH, OMIM: #143890), lead to about two percent of early myocardial infarction (MI) [1]. FH is one of the common lipid disorders with an approximate prevalence of 1:250 [2]. It has autosomal dominant inheritance [3] although there is a low frequency of autosomal recessive pattern with an estimated prevalence of 1 in 200,000 to 300,000 [4,5]. The frequency of FH varies among different populations and a proportion of affected individuals remain undiagnosed [6]. There is no study about the prevalence of FH in Asia. It seems that the prevalence may differ because of sociodemographic characteristics.

FH has a wide spectrum of clinical symptoms, due to defects in proteins involved in LDL uptake and catabolism [7], including the proteins encoded by LDL-receptor (*LDLR*), apolipoprotein-B (*APOB*), LDL receptor adaptor protein (*LDLRAP1*) and PCSK9 (*PCSK9*) [4]. More than 90% of FH cases are due to pathogenic variants in the *LDLR* gene

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[8]. Pathogenic variants in *LDLR* lead to an abnormal increase in low-density lipoprotein cholesterol (LDL-C) resulting in 5–8 times higher risk of premature coronary artery disease (CAD) [9]. Xanthomas, premature and progressive atherosclerotic cardiovascular disease (ACVD) are seen in the patients [8].

To date, approximately more than two thousands pathogenic variants have been reported in *LDLR* in HGMD professional 2019 (http://www.hgmd.cf.ac.uk), including different types of variants i.e. missense, nonsense, large deletion, duplication, indel, regulatory and splicing mutations. The type of variant could affect the degree of increased LDL and cholesterol, severity of the disease and risk of CAD development [10]. Different types of variants have been reported among various Asian countries and we aim to investigate the distribution, frequency, functional variants, zygosity, and clinical phenotype in Asia. Only the developed Asian countries have a systematic review of the variants in their population. The aim of this systematic review is to explore all records of FH *LDLR, APOB* and *PCSK9* mutations reported in databases for Asia as the major genetic causes of monogenic FH. In patients with no causative mutation, the polygenic cause is suggested, which is out of the scope of this manuscript. In addition, the genotypic spectrum of the Iranian population is studied and reported in this survey to add new data on *LDLR* mutations to Asia.

2. Patients and methods

2.1. Search strategy

A systematic search was conducted on the Asian published articles about familial hypercholesterolemia patients with *LDLR, APOB* and *PCSK9* pathogenic variants. All the English published articles in databases (PubMed, Science Direct, John Wiley, Google Scholar) were searched from 1950 to 2019 using the following keywords: *LDLR* [title] gene mutations OR *LDLR*, AND familial hypercholesterolemia. *LDLR* and name of each Asian country were specifically used to find all the relevant articles, for example IRAN AND *LDLR* (Fig. 1). The MeSH term for PubMed is available (Supplementary MeSH). Also, *APOB and PCSK9* variants were searched using terms "PCSK9" AND "gene mutation" AND "familial hypercholesterolemia" and "*APOB*" AND "gene mutation" AND "familial hypercholesterolemia".

To note, Asian countries were classified according to https://www.countries-ofthe-world.com/countries-of-asia.html and www.worldometers.info/geography/how-many-countries-in-asia/ (Supplementary countries).

2.2. Study selection

The articles were selected according to the following inclusion criteria: 1) original, cohort, cross sectional, case-control, case-report studies, 2) patients clinically diagnosed with FH having *LDLR, APOB, PCSK9* mutations and 3) patients with Asian ethnicity.

The non-English articles, congress abstracts, editorials, letters, books, reviews, systematic reviews, non-Asian studies, *in vitro* and *in vivo* studies, functional studies and pharmacogenomic studies, other genes/mutations involved in FH were excluded; furthermore, articles with insufficient data were not included in the study.

2.3. Data extraction

The accuracy and validity of the selected articles were reviewed by investigators. The title and abstract of each article were reviewed independently considering inclusion criteria. Number of patients/families, clinical significance of the variants, population, zygosity, low-density lipoprotein cholesterol (LDL-C) level, total cholesterol (TC), triglyceride (TG) level, high-density lipoprotein (HDL) level, nucleotide and amino acid change and type of molecular technique were extracted from the articles. Nomenclature for the description of sequence variants was applied to all genetic entries according to the Human Genetic Variation Society (HGVS) recommendations (http://varnomen.hgvs.org/). Entries with unknown or uncorrected naming were removed or corrected to ensure the adherence to HGVS. Also, authors were

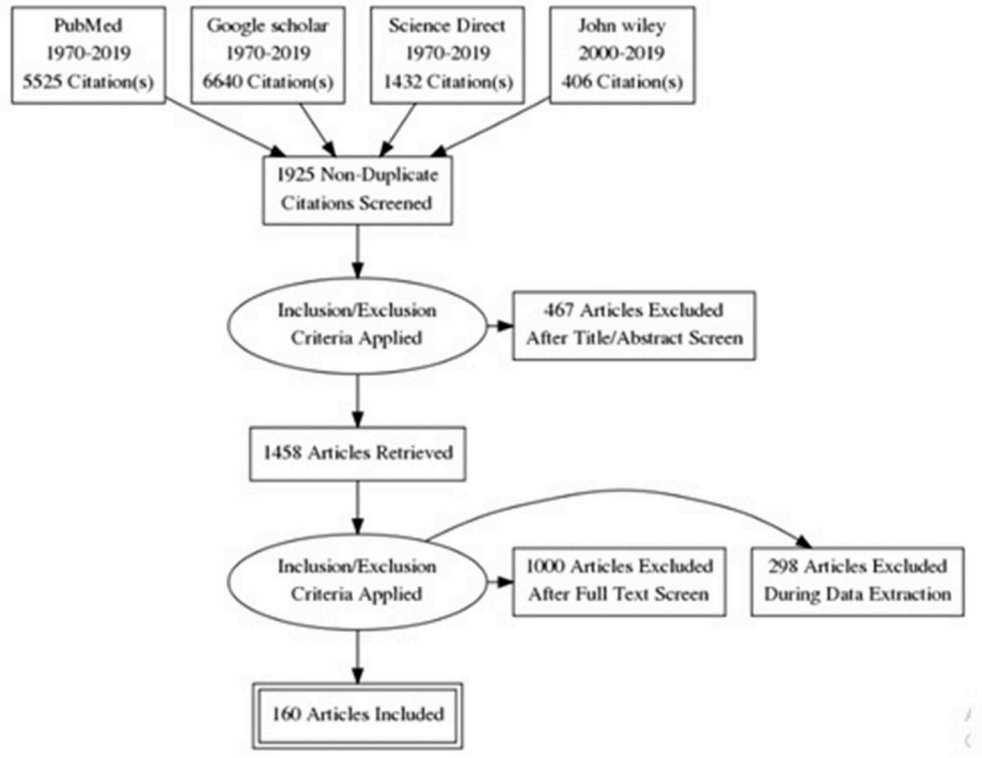

Fig. 1. Study selection process including databases, number of articles, and final number of included articles. The relevant articles with *LDLR* mutations have been available since 1970.

contacted for additional information.

2.4. Quality assessment

The American College of Medical Genetics and Genomics (ACMG) guideline was used to assess the quality of the variants [11]. The studies were selected based on inclusion criteria. The studies enrolled were selected based on Newcastle-Ottawa Scale (NOS) (http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp) to evaluate the quality of data; studies rating ≥5 (out of 9) were selected for this study. Also, quality assessment of variants was conducted by two investigators. The quality of the studies was discussed between investigators and the final decision was reached with consensus.

This systematic review was conducted following preferred reporting items or systematic reviews and meta-analysis (PRISMA 2009) statement.

2.5. Statistical analysis

Data were analyzed with the statistical Package for Social Sciences (SPSS version 22.0, SPSS, Inc., Chicago, Ill, USA).

2.6. Study participants and molecular analysis of LDLR gene

The clinically diagnosed individuals with FH were referred to the genetic laboratory. Biochemical characteristics such as TC, HDL, LDL-C, and TG were reordered for each patient. After obtaining signed informed consent, genetic testing was performed for 37 cases clinically diagnosed with FH. The methods used for patients are in accordance with the genetic testing guidelines for familial hypercholesterolemia and all experimental protocols were approved by the Rajaie Cardiovascular Medical and Research Center.

2.7. In silico analysis

The gene reference sequence NG_009060, NM_000527.4 was used to determine the variant position. Position of the variants in protein was determined based on UniProtKB/SwissProt P01130. The functional effect of each variant was predicted based on amino acid domains and regions of LDL-R protein.

2.8. Ethical approval

The experimental protocols are approved by Rajaie Cardiovascular Medical and Research Center, genetic testing service, Iran University of Medical Sciences.

3. Results

3.1. Search analysis

The search strategy yielded 14,003 articles since 1970. Duplicates were removed and 1925 articles remained. These articles were reviewed according to inclusion and exclusion criteria. 1458 articles remained only based on title and abstract; 1000 articles were excluded after review of published literature considering the criteria. After data extraction, 160 eligible articles were included in our systematic analysis (Fig. 1). Any entry that could not be verified was deleted from this study.

Among 48 different countries in Asia, 20 countries published articles on *LDLR* mutations. China, Japan, India and Taiwan constituted 68% of published articles (Table 1 and Supplementary Tables S1, S6, S7, S8). 8994 families were investigated in the Asian population (Table 1). Japan and China represented 19% and 27% of the publications, respectively. A total of 629 mutations were reported from Asian ethnicities (Supplementary Tables).

Table 1
Published articles, number of families, reported *LDLR* variants in Asian countries according to search strategy.

Asia regions[a]	Publications	Families	Variants
North and South East	8	658	54
South	15	357	51
East	90	5476	496
West	21	672	40
Central	0	0	0
Multi-ethnicity	10	1277	121
Trans-continental countries	15	554	101
Total	160	8994	629 (non-duplicate variants)

[a] The classification of Asian regions in shown is Supplementary Data.

The clinical phenotype and biochemical assays such as TC, TG, LDL-C, and HDL for each population and variant were extracted from publications (Supplementary Tables). The clinical phenotype of the study varied including 196 xanthomas, 152 CAD, 32 MI, 10 angina, 35 corneal arcus and others (17) were defined as cardiovascular diseases. The mean TC and LDL-C level for each population was determined (Table 2). Mean TC was > 7.9 mmol/L and mean LDL-C was > 5 for heterozygotes in different populations. All the studies had LDL-C levels > 13 mmol/L for homozygotes except Taiwanese (8.4 mmol/L) and Arabians (12.4 mmol/L) (Table 2). Accordingly, the homozygous genotypes had higher LDL-C levels than heterozygous patients.

The variants were counted based on the number of variants in different published articles; 27 mutations were distributed sequentially along the Asian countries based on frequently published variants (Supplementary Tables).

Accordingly, the variants were 312 missenses (50%), 67 nonsenses (11%), 119 frameshifts (19%), 46 copy number variations (small and large deletions) (7%), and 56 splicing (9%) variants. Also, synonymous substitutions accounted for 11 of 629 variants with unknown pathogenicity. Nonsense and frameshift variants were predicted as loss of function variants, pathogenic/likely pathogenic variants; this included 37% of the variants. Splicing (9%) and regulatory variants (4%) were predicted to cause haploinsufficiency. Also, missense variants may cause haploinsufficiency; as we cannot predict the functional effect of the missense variant unless *in vitro* analysis is performed to confirm the effect.

The distribution of the *LDLR* variants in Asia included 202 (40%) ligand binding site class A, 165 (33%) ligand binding site class B, 92 (18%) epidermal growth factor domain, 13 of 507 O-linked sugar domain, and transmembrane and cytoplasmic domains.

Different methods of detection of the variants have been used for genetic analysis of FH in different populations (Supplementary Tables). 31% of the variants (50 of 160) were investigated only by direct sequencing in the population. NGS and targeted exome sequencing was used in 11 of 160 (7%) of the studied publications. Furthermore, 62% of mutations were analyzed by combination of genetic techniques e.g. RFLP and Sanger sequencing.

The zygosity was determined for the defined number of individuals in different populations (Table 2). The frequency of the variants in various populations was investigated and frequent variants were determined for each as indicated below.

3.2. LDLR mutations in South East Asia

In total, 8 articles were found in which 54 different mutations were determined (Table 1 and Supplementary Table S9). A high number of articles were published from Thailand in comparison to other countries of this region; Singapore had 77% heterozygosity rate among South East Asian countries (Table 2 and Supplementary Table S9). Only two homozygous variants were reported from Vietnam. Malaysia had 9

Table 2
The biochemical analysis of patients in different Asian populations. Heterozygote, homozygote, compound heterozygote distribution among countries.

Country (no of cases)	Number of individuals with available biochemical data	Total cholesterol (mmol/L)	Triglycerides (mmol/L)	LDL-C (mmol/L)	HDL-C (mmol/L)
North and South East Asia					
Thailand (2)	2	10.2	2.6	8.2	1.4
Het (2)	2	10.2	2.6	8.2	1.4
Malaysia (164)	164	7.9	1.9	5	1.3
Het (43)	NA	NA	NA	NA	NA
Singapore (56)	50	8.5	1.2	6.7	1.4
Het (51)	50	8.5	1.2	6.7	1.4
Hom (5)	NA	NA	NA	NA	NA
Vietnam (4)	4	18.4	NA	16.2	NA
Hom (4)	4	18.4	NA	16.2	NA
South Asia					
Pakistan (5)	5	15.4	1.0	12.3	1.1
Het (5)	5	15.4	1.0	12.3	1.1
India (31)	19	14.6	2.9	12.2	1.04
CH (3)	3	18.1	1.5	16.4	0.6
Het (16)	6	8.2	5.2	5.2	1.2
Hom (12)	10	17.4	2.2	15.1	1.1
Sri Lanka (4)	4	10.9	NA	7.7	NA
CH (1)	1	13	NA	10.8	NA
Het (3)	3	9.4	NA	6.98	NA
East					
China (513)	177	13.01	1.4	11.4	1.2
CH (91)	69	11.01	1.3	8.7	1.2
Het (134)	63	9.42	1.6	8.2	1.1
Hom (70)	45	16.5	1.3	13.6	1.3
(218)[a]	218	13.2	1.3	11.03	1.5
Japan (797)	122	14.21	1.8	11.65	1.3
CH (30)	28	15.3	1.5	13.22	0.97
Het (719)	46	9.95	1.72	7.58	1.40
Hom (48)	48	17.4	2.24	14.14	1.49
[a]	Unknown	8.08	1.50	6.95	0.74
Korea (106)	94	8.54	1.7	6.15	1.45
Het (38)	38	8.98	1.6	7.4	0.9
Taiwan (721)	48	12.5	1.25	9.44	1.1
CH (23)	15	15.9	NA	12.98	1.15
Het (271)	31	10.6	1.3	8.02	1.3
Hom (3)	2	16.5	1.2	8.4	0.8
(440)[a]	440	8.98	1.46	6.54	1.39
West					
Lebanon (133)	–	NA	NA	11.5	NA
CH (0)	0	–	–	–	–
Het (121)	15	NA	NA	8.2	NA
Hom (62)	19	NA	NA	14.0	NA
Arab[b] (17)	17	15.1	1.2	12.1	0.9
CH (1)	1	21.3	2.5	18	0.9
Het (4)	4	8.8	1.8	7.1	0.96
Hom (12)	12	15.2	1.1	12.4	0.8
Iran (135)					
Het (1)	0	–	–	–	–
Hom (9)	9	18.4 ± 4.6	NA	13.6 ± 3.8	NA
[a]	125	8.5	1.4	5.2	1.3
Het (6)	6	9.3	1.33	6.29	1.46
Hom (12)	12	15.4	1.92	13.2	1.49
Total mean (18)	18	13.3	1.73	10.89	1.48
Israel (103)					
CH (1)	1	12.7	NA	11.4	0.9
Het (102)	20	7.99	1.7	5.9	1.2
Hom (0)	0	–	–	–	–
Multi-ethnicity (247)	30	9.8	1.3	7.05	1.7
CH (1)	1	NA	NA	NA	NA
Het (27)	27	9.5	1.3	7.1	1.7
Hom (2)	2	18.3	NA	NA	NA
Turkey (32)	32	13.9	1.2	11.4	1.3
CH (5)	5	15.4	1.02	12.3	1.1
Het (12)	12	8.02	1.4	5.8	1.3
Hom (15)	15	18.1	0.98	15.6	1.4
Russia (42)	42	10.8	12.5	8.99	1.2
CH (3)	2	11.7	1.06	9.7	1.6
Het (39)	27	10.8	1.7	8.96	1.9
Hom (0)	0	–	–	–	–

Hom: homozygote, Het: heterozygote, CH: compound heterozygote, NA: not available.
[a] Publications including only biochemical assays and total mean biochemical assays.
[b] Arab including Saudi Arabia and Oman.

Table 3
The distribution of the common variants in Asian countries.

no	Mutation name	Protein change	Total no. of articles	Southeast Asia			Western Asia				South Asia	East Asia				Cyprus	Russia	Multi-ethnicity	Ref
				Malaysia	Philippine	Singapore	Turkey	Iran	Lebanon	Israel	India	China	Taiwan	Japan	Korea				
East Asia																			
1	c.2054C > T	p.Pro685Leu	22			1				1	2	9	2	43	7			1	[12]
2	c.1747C > T	p.His583Tyr	18		1	9						18	41		3				[13]
3	c.1448 G > A	p.Trp483Ter	17									36	3						[14]
4	c.1702C > G	p.Leu568Val	5											23	1				[15]
5	c.2141-?_2311 + ?del	—	2			1								16					[16]
6	c.986G > A	p.Cys329Tyr	13		1	2						9	31						[17]
7	c.1246C > T	p.Arg416Trp	15				1	1		1		4	9	4	2			1	[18]
8	c.268G > A	p.Asp90Asn	15		1		1					3	21		2			1	[18]
9	c.1765G > A	p.Asp589Asn	13			4						8	8		3				[19]
10	c.682G > A	p.Glu228Lys	13							1	1	3	3	10	4			1	[20]
11	c.682G > T	p.Glu228Ter	13									4	2		7		1	2	[21]
12	c.1879G > A	p.Ala627Thr	12									14	6						[13]
13	c.1432G > A	p.Gly478Arg	10									3	11					2	[21]
14	c.313+1G > A	—	7			1						14					2	6	[22]
15	c.1322T > C	p.Ile441Thr	5									2	13						[14]
West Asia																			
16	c.2043C > A	p.Cys681Ter	9						103	22						7		6	[23]
17	c.1171G > A	p.Ala391Thr	3	1					10			1							[24]
18	c.2479G > A	p.Val827Ile	2							8							1		[21]
19	c.1729T > C	p.Trp577Arg	4				8	3											[25]
South East																			
20	c.301G > A	p.Glu101Lys	4	11										1				7	[26]

variants in 43 patients with the heterozygosity rate of 26% (Table 2 and Supplementary Table S10). The frequent variants in this geographical region were c.301G > A (p.Glu101Lys, 11 of 43), c.763T > A (p.Cys255Ser, 10 of 43) and c.601G > A (p.Glu201Lys, 9 of 43) in Malaysia (Supplementary Table S9E). 43 variants were found in Singapore with the missense variant c.1747C > T (p.His583Tyr, 9 out of 66) being frequent (Supplementary Table S9F). Vietnam and Philippine had no frequent variants.

Biochemical assays were used for variants and patients in each population. The clinical phenotype was determined for some patients (Supplementary Tables S9E and F).

3.3. LDLR mutations in South and Central Asia

Eight countries located in South Asia. Four countries published articles on *LDLR* mutated FH patients. Overall, 51 mutations were found in South Asian countries. India had ten (47%) published articles, and 39 unique variants (Supplementary Table S4). No relevant published articles were found in Central Asia.

India had a 39% homozygosity rate (Table 2). No frequent variant was found for India, Sri Lanka and Pakistan (Table 3 and Supplementary Table S4A, S3 and S9). Clinical phenotype and biochemical assays were determined for the population of these regions (Table 2, Supplementary Tables S4B, S3, and S9D).

3.4. LDLR mutations in West Asia

In 9 out of 13 West Asian countries, no article on *LDLR* gene analysis was found. 33 different mutations in *LDLR* were found in the remaining countries. Ten mutations were reported in Saudi Arabia and 1 mutation in Oman (c.272delG) (Supplementary Table S5). Iran and Lebanon each published 4 articles and had 10 and 17 different mutations, respectively (Supplementary Table S9). Three articles were found in Israel and 34 mutations were found in these studies.

The homozygosity rate was high in Lebanese 34%, and Arabians 70%. The heterozygosity was high in Israel (Table 2).

The frequent variants investigated were as follows: c.1729T > C (p.Trp577Arg, 3 of 10) in Iran, c.2027delG (p.Gly676Alafs*33, 7 of 17) in Arabia, c.del197 (p.Val66Hisfs*63, 35 of 103 (34%)) in Israel, and c.2043C > A (p.Cys681Ter, 103 of 183) and c.1171G > A (p.Ala391Thr, 10 cases) in Lebanon (Supplementary Tables S9 and S9B). The heterozygosity rate of c.2043C > A (p.Cys681Ter) variant was 56%, known as Lebanese allele. Its frequency was 21% in Israel, its neighboring country (Table 2).

The clinical phenotype and lipid profile were identified for each study (Supplementary Table, S5B, S9B, C and G).

3.5. LDLR mutations in East Asia

Our data revealed 408 mutations in East Asia, which accounted for 65% of the total reported mutations. Our study showed that frequent mutations were mainly located in East Asia, because the main publications are reported in this region (Table 1).

Japanese studies showed 172 variants in 797 cases. 719 (90%) were heterozygotes; homozygotes accounted for 48 (6%) variants. Totally, 346 variants were found in this population. The frequent mutations in Japan were c.2431A > T (p.Lys790Ter), 2312-3C > A, c.1845+2T > C, c.1012T > A (p.Cys338Arg), and c.1297G > C (p.Asp433His) accounted for 241 (30%), 47 (6%), 32 (4%), 26 (3%) and 21 (3%) variants, respectively (Supplementary Table S6A).

295 cases, including 134 heterozygotes, 70 homozygotes and 91 compound heterozygotes, were found in the Chinese population. Totally, 177 mutations were found in the Chinese population. The frequent variant is c.1448G > A in China (Tables 2 and 3, and Supplementary Table S1A).

271 out of 297 cases were heterozygotes in Taiwan (Supplementary Tables S8A and B). 81 variants were detected in this population, in which 1747C > T (p.His583Tyr) was the frequent variant (Table- 3). The heterozygosity rate in Korea was 36%. Among 50 variants, c.661G > A (10%) was the frequent variant in Korea (Supplementary Table S7A).

The clinical and biochemical assays are indicated for each population (Supplementary Tables S1B, S6B, S7B, S8B).

3.6. LDLR mutations in patients with multi-ethnicity

Among 160 published articles, 10 articles enrolled patients from at least two different ethnicities. Despite a few numbers of studies (10 articles), 121 different mutations were reported among these patients, which accounted for 19% of total reported mutations (Supplementary Tables S10A and B). Among the 30 individuals, 2 were homozygotes, 1 compound heterozygote and 27 heterozygotes (Table 2).

3.7. LDLR mutations in transcontinental countries (Turkey, Russia, Cyprus)

Turkey is a country located mainly in Western Asia. This study showed that 7 articles have been published in Turkey; 28 different mutations were determined in this region. Russia is another transcontinental country in the Northern part of Asia. We identified 39 mutations in patients with Russian ethnicity (Table 1 and Supplementary Tables S2 and S9). Our search strategy revealed one study in Cyprus. 4 different mutations were determined in the Cyprus study (Supplementary Table S9).

15 of 32 individuals were homozygotes and 12 were heterozygotes in Turkey. The frequent variant was c.1729T > C (p.Trp577Arg) in Turkey and c.2479G > A (p.Val827Ile) in Russia (Supplementary Table S2A and B, S9A and H). The frequent variant was c.190+4A > T, found in 3 cases in Cyprus (Supplementary Table S9D).

3.8. Common variants in Asia

The frequency of the variants was determined for each population. The common variants were identified between the neighboring populations and other Asian countries (Table 3).

The common variants were c.301G > A (p.Glu101Lys) in Malaysia, c.1747C > T (p.His583Tyr) in Singapore, c.2043C > A (p.Cys681Ter) in Lebanon and Israel. The variant c.1171G > A (p.Ala391Thr) in Lebanon was also seen in Malaysia and China. c.1729T > C (p.Trp577Arg) was also common between Iran and Turkey (Table 3).

43 of 797 cases had c.2054C > T (p.Pro685Leu) change in Japan, which was also seen in China (9 of 295), Korea (7 of 38), and Taiwan (2 of 297). China had 1448G > A (p.Trp483Ter) in 36 of 295 (12%) individuals of the population seen in Taiwan. 41 of 297 (14%) Taiwanese had c.1747C > T (p.His583Tyr) mutation, which accounted for 6% of patients in the Chinese population and 16% in the Singapore population (Table 3).

3.9. APOB and PCSK9 mutations in Asia

Our study showed 20 studies in Asia with 41 *APOB* variants after duplicate removal (Supplementary Table S10). 184 heterozygous individuals carrying *APOB* variants were detected and no homozygous variant was found. *APOB* gene included 58 variants in Taiwanese, 47 in Chinese, 27 in Vietnamese, 17 in Arabs and the rest were found in Singapore, Korea, Israel, and Japan. The most common variant of *APOB* was p.Arg3527Trp, accounting for 78 of 184 heterozygous variants. Of course, 27 Vietnamese carried p.Arg3527Trp and 40 cases carried this variant in Taiwanese (Supplementary Table S10).

20 variants of *PCSK9* were found in 18 studies. Totally, 160 patients were found including 117 heterozygotes and 43 homozygotes (Supplementary Table S11). The most common variant was p.Glu32Lys

in the Asian population seen in Japan.

To note, there is lack of information from most of the countries in Asia and further investigation is needed to draw a conclusion about the frequency of these variants in FH.

3.10. Genetic testing in the Iranian population

Molecular analysis of 37 patients determined 18 variants in the studied cases. Seven novel variants were reported in our studied population. The variants included seven missense mutations, 5 nonsenses, 4 frameshifts (2 deletions and 2 insertions) and two splicing variants. Twelve were homozygotes and 6 variants were heterozygotes (Supplementary Table S14). Therefore, 12 of 18 had LOF, known as "negative LDLR". Two splicing variants were homozygote; four missense variants were also homozygous; in addition, two termination variants were heterozygotes, but we predicted them as "defective LDLR" because of functioning in a multi complex receptor (Supplementary Table S14).p.Ser130Ter was common in this study. Two nonsense heterozygous variants were defined as pathogenic. The splicing variant at intron 15 was determined in one case. Previous reports have shown c.2312–2A > C mutation that causes alternative splicing and results in deletion of exon 16 [27] (Supplementary Table S14).

3.11. Genotype-phenotype correlation

Biochemical characteristics of each patient in the Iranian population were determined (Supplementary Table S14 and Table 3); their relation to the genotype was investigated. The mean age of the patients with known genotype is 31.1 ± 14.02 years; with mean plasma cholesterol level of 516.5 mg/dL (13.3 mmol/L). The mean LDL-C value of the patients was 421.3 mg/dL (10.9 mmol/L), the mean high density lipoprotein (HDL) value 57.2 mg/dL (1.5 mmol/L), and mean triglyceride (TG) 153.7 mg/dL (1.7 mmol/L) (Table 2).

The mean cholesterol level for homozygous patients was 595.17 mg/dL (15.4 mmol/L) and LDL-C value was 510.17 mg/dL (13.2 mmol/L), mean HDL 57.83 mg/dL (1.5 mmol/L) and mean TG 171.33 mg/dL (1.9 mmol/L). The mean criteria of FH diagnosis was higher in homozygous in comparison to the total values (Table 2).

The heterozygous variants having termination effect on receptor had low LDL-C (210 and 140 mg/dL) and (total cholesterol) TC values (320 and 206 mg/dL). Therefore, these are suggested to cause an LDL defective receptor. Similarly, it is concluded that the other four heterozygotes may cause LDL defective receptor having haploinsufficiency (Supplementary Table S14).

4. Discussion

To the best of our knowledge, this is the first systematic review of *LDLR, APOB, PCSK9*-related FH patients within Asian countries. To date, only few systematic reviews were conducted on the *LDLR* gene mutations in China, Arabic and Latin American countries [28–30]. We gathered and analyzed a comprehensive panel and geographical distribution of mutations in monogenic FH patients reported in Asian countries. The polygenic influence of the mutations in HeHF was not studied in this systematic review.

Briefly, in our systematic review 629 variants among 8994 families were recorded from 20 countries. Approximately, 42% of the Asian countries did not have any information about FH.

The quality of variants was based on ACMG and pathogenic variants were selected as genetic diagnostic factors for FH. Estimation of the clinical phenotype, zygosity, functional variants and frequency of the variants was included based on the reported participants in each country and any bias may be caused by errors in clinical diagnosis, reporting variants, outcome measurement, and statistical analysis of the study.

Our results indicated that there is a significant genetic heterogeneity among FH affected patients. Missense mutations accounted for approximately 50% of the variants. CNV (deletions, large duplications, large deletions) accounted for 46 of 629 (7%) of the reported variants in Asia. To explain, copy number variants account for > 10% of pathogenic variants in founder mutations [31]. Therefore, for diagnostic purpose, CNV analysis should be considered for genetic testing not to miss any causal variant.

Only < 15% of the *LDLR* variant pathogenicity has been functionally analyzed *in vitro* [32]. Functional analysis from literature and prediction analysis of the variants (online database) revealed that 326 of 629 pathogenic/probably pathogenic variants in *LDLR* gene are present. Of course, 30% (186 of 629) are nonsense and frameshift variants. It is assumed that nonsense, frameshift variants, and CNVs cause truncated LDL receptor and are pathogenic. They produce no protein or non functional receptors and are categorized as "receptor-negative". Missense variants are predicted to cause both "receptor-negative" and "receptor defective" proteins. Therefore, careful interpretation of the results is needed; also functional studies provide information about the pathogenicity. The presence of false positive and false negative reports may be obvious in this systematic review. Among the reported variants, 73% were located at the ligand binding domain.

The twenty seven mostly reported variants were investigated in the *LDLR* gene (Supplementary Table S11). The first ones are p.Pro685Leu, p.His583Tyr, and p.Trp483Ter (Supplementary Table S11). In comparison, they were also common in the Chinese population [28]. In addition, Arabic studies demonstrated that among these 27 frequent mutations c.2043C > A (p.Cys681Ter) was frequent mainly in Lebanon [29]. Variant p.Cys681Ter, known as Lebanese mutation, identified in 1987 by Lehrman, accounts for 82% and 45% of HeFH and HoFH Lebanese patients, respectively [33].

Twenty common variants were determined in the *LDLR* gene in Asia, and the distribution was shown in other countries. The frequency of the variants was higher among neighboring countries (Table 3). Some variants have a higher frequency in one specific region due to the founder effect. For instance, p.Pro685Leu is the most common variant in Eastern Asia. Likewise, p.His583Tyr is common in Taiwan, China, Korea and Singapore, which may reflect immigration among these countries. In the same way, the *APOB* and *PCSK9* common variants show the same pattern.

We have included genotypic data in the Iranian population due to the fact that some of our variants reported for the first time can complete the list of *LDLR* mutations reported worldwide and in Asia. Interestingly, our investigation revealed a high frequency of *LDLR* gene homozygosity (67%) in this population in comparison to other studies. These differences are the consequence of consanguineous marriages. Other patients may have variants in other genes or have a polygenic basis of the disease, which should be investigated in future studies. The French Canadian Québec population, Lebanese, South Africans have higher homozygous frequency due to isolation and consanguineous marriages. A genetic drift may occurred in the Quebec population [34].

Notably, HoFH patients with *LDLR* negative (< 2% activity) have higher LDL-C level than *LDLR* defective (2–25% activity) patients [7]. Homozygous variants in the studied patients have higher mean LDL-C 13.2 mmol/L (510 mg/dL) value and the mean cholesterol level of 15.4 mmol/L (595 mg/dL). TG is also higher in homozygous (1.9 mmol/L or 173 mg/dL) compared to all (1.7 mmol/L or 153 mg/dL). This is also true in other Asian countries and the mean LDL-C level of homozygous patients is > 13 mmol/L in comparison to the guidelines. The zygosity analysis shows correlation to the severity of the phenotype and LDL-C level (Table 2).

Increased LDL-C levels in FH patients are due to impaired LDL-receptor activity, which is caused by different classes of *LDLR* mutations. This study brings information about the reported variants of the studied populations in Asia. According to the ACMG guidelines and the extracted Asian variants, 46% are annotated as pathogenic/probably

pathogenic. The problem is that there are variants with unknown significance of pathogenicity for which *in vitro* analysis or family studies are required to define the variant. Population based reports of the variants may give geneticists and clinicians clues about the influence of the variants.

Our study limitations include restriction of other language usage and data extraction from non-English published cohort studies conducted in Asian countries. Those articles not having the search terms would have been missed through the data extraction step. Besides, data from some of the published articles were insufficient for data extraction e.g. biochemical assays, and zygosity. The clinical data were not available for all the studies, so we could not find out about the genotype and phenotype correlation of the variants. The distribution of the variants could not be investigated because there were not enough sampling studies. The frequency and prevalence of variants are not defined due to the lack of information in some publications. If more large-scale studies are performed, genotype and phenotype could be determined in Asia. To note, the pathogenicity of the variants was based on *in silico* analysis, and functional analysis is needed to determine the functional influence of missense variants.

In conclusion, this is the first systematic review of *LDLR* mutations in FH patients in Asia. The identified variants provide a valuable source for physicians and researchers in this part of the world. Although, the Western part of Asia has limited studies, we encourage research to investigate FH in this part of Asia. This study provides information for health care system providers to be aware of the signs and diagnosis of FH and benefit from early treatment in Asia. Unraveling common variants in each country could prioritize cost benefit diagnostic programs and future probable therapeutic strategies. Furthermore, a specific lifestyle could help patients with early diagnosis provided by cascade screening.

CRediT authorship contribution statement

Nejat Mahdieh: Validation, Project administration, Writing - review & editing. **Katayoun Heshmatzad:** Validation, Writing - original draft, Writing - review & editing. **Bahareh Rabbani:** Writing - review & editing.

Declaration of competing interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2020.05.004.

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